

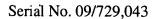


REMARKS

No new matter has been added by virtue of this Amendment and the accompanying Substitute Sequence Listing. The change to the sequence on page 21, line 15, corrects a typographical error. This correction is based on the sequence disclosed by SEQ ID NO:12 in the originally filed sequence listing. Therefore, no new matter is introduced. The remaining amendments to the specification only pertain to the insertion of SEQ ID NOs.

Also submitted with this Amendment is a Substitute Sequence Listing containing SEQ ID NO:1 through SEQ ID NO:13, on both paper and diskette, and a "Statement to Support the Filing and Submission of the Sequence Listing in accordance with 37 C.F.R. §§1.821-1.825."

Attached hereto is a marked-up version of the changes made to the claims and specification in this amendment. The attached pages are captioned "<u>Version with Markings to Show Changes Made</u>".







AUTHORIZATION

No additional fee is believed to be necessary. The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 4167-4000. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: Dorothy R. Auth

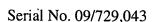
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APPENDIX: Version with Markings to Show Changes Made (Additions are indicated by underlines, deletions are indicated by brackets)

The paragraph beginning on page 16, and ending at line 2, page 17, has been amended as follows:

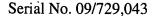
The YACs to be targeted were obtained from the Princeton mouse genomic YAC library (6). Isolation and characterization of the Hoxb and Hoxc cluster YACs have been previously described (7, 8). The targeting vector designed to clone the Hoxc-8 gene, pClC9C6, was made as follows. primers for the Hoxc-6 gene (9) were designed to the 5' untranslated region of the gene. The 5' primer was: 5'-TAGATCTGTTTGTCTCCCACATGCC-3' (SEQ ID NO:1) and contained a Nrul linker. The 3' primer was: 5'-AGGTGGCAGGATAAGGAAGGGTTAG-3' (SEQ ID NO:2) and contained a HindIII linker. The product was 706 bp. The PCR primers for the Hoxc-9 gene (10) were designed to amplify a 624 bp fragment of the 3' untranslated region. The 5' primer was: 5'- CGACAAGGAACAAATCCTAAGCCC-3' (SEQ ID NO:3) and contained a BamHI linker. The 3' primer was: 5'-TGCATTTGCAGCCTGATCCAGCCA-3' (SEQ ID NO:4) and contained a NruI linker. All polymerase chain reactions (PCR) was carried out in a Hybard Omnigene machine. Reactions contained 1X Boehringer Mannheim PCR buffer supplemented with 2.5 mM MgCl₂, 0.8 mM dNTPs and 0.5 U Tag Polymerase (Boehringer Mannheim). The Hoxc YAC was used as template. Reaction conditions were 94° 30s, 55° 30s and 72° 45s for 30 cycles. PCR products were cloned into the Ttailed vector, pCRII (Invitrogen Corporation). BamHI-NruI PCR fragment was excised and ligated head-to-tail at the NruI site of the cloned Hoxc-6 PCR fragment. The 1.4 kb BamHI/HindIII fragment containing the two PCR products was isolated and ligated to pCLASPER digested with the same enzymes to create pClC9C6.



The paragraph which begins at page 17, line 2, has been amended as follows:

The targeting vector designed to clone the entire insert from a Hoxb cluster YAC, pClYA, was made by overlap PCR as follows. PCR primers were designed to the YAC arms of pYAC4 (11). One set of primers were designed to amplify a region of 484 bp upstream of the single E. coli site of pYAC4. The 5' primer was: 5'-TCTCATGTTTGACAGCTTATCA-3' (SEQ ID NO:5) (position 1-23 of pYAC4 in GenBank accession number U01086) and contained a HindIII linker. The 3' primer was: 5'-AGAGTATACTACATAACATAACACA-3' (SEQ ID NO:6) (position 460-484) and contained an NruI linker and a 15 bp overlap with the 5' primer for the downstream pYAC4 sequence. The second set of primers were designed to amplify 494 bp of pYAC4 downstream of the E. coli site. The 5' primer was: 5'-TTCAAGGGAATTGATCCTCTACG-3' (SEQ ID NO:7) (position 656-678) and contains an NruI linker and 15 bp of sequence designed to overlap with the 3' upstream primer described above. The 3' primer was: 5'-AAGATTCCGAATACCGCAAGC-3' (SEQ ID NO:8) (position 1130-1150) and contained a BamHI linker. The Hoxb YAC was used as template in PCR conditions which were as described above with the following modifications. After 10 cycles, part of each reaction was diluted 1:10 and 1 μ l of each was added to a new reaction containing primers for the 5' upstream and 3' downstream primers. PCR was continued for an additional 25 The 975 bp product containing both PCR fragments with an NruI site in-between was cloned into pCRII and subsequently digested with BamHI and HindIII and ligated to pCLASPER to create pClYA.

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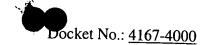




The paragraph which begins at the last line of page 20 and ends at line 25, page 21, has been amended as follows:

Whole cell PCR was performed on yeast colonies to identify recombinants. Individual yeast colonies were first replica plated by streaking in patches onto drop-out media plates lacking leucine. A generous portion of each replica patch was resuspended in 100 μ l of sterile water. 10 μ l of resuspended yeast was used as template. The primers used to detect recombinants for pClC9C6 were: 1) primer pair in which one primer is specific to the LEU2 region of pCLASPER, 42 bp proximal to the start of the polylinker and the second is specific to the Hoxc-6 5' untranslated DNA in the insert vector primer 5'-TTAAAGAACGTGGACTCCAACG-3' (SEQ ID NO:9). Hoxc-6 primer 5'-ACTGTGCTCTGCAGTCTCATCCG-3' (SEQ ID NO:10). Primers specific to the second exon of Hoxc-8.5' primer 5'-CGCAGCGGTCGACAAACTTACA-3' (SEQ ID NO:11); 3' primer 5'-CTCCTCCTCTTCCTCTTCCTA-3' (SEQ ID NO:12). The primers used to detect recombinants for pClYA were: 1) Vector primer described above with primer specific to pYAC4 in the insert pYAC4 primer 5'- CAACTTGGCTACCGAGAGTA-3' (SEQ ID NO:13) (position 501-520 in pYAC4 in GenBank); and 2) primers specific for the Hox-2.9 gene (Hoxb-1; 7). PCR conditions were as described above except that a 5 minute 94°C cycle was added prior to the amplification cycles to lyse the yeast cells and 45 amplification cycles were used. When total yeast DNA or bacterial minipreps were used as template, PCR conditions were as described in the construction of the vector.





AUTHORIZATION

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